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/THE METABOLISM OF LINOLENIC ACID/

by

PATRICIA MURPHY RANDOLPH

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INTRODUCTION

Burr and Burr (5) showed that rigid dietary restriction of fat produced a typical dermatitis and retarded growth. As the addition of either linoleic or linolenic acids to the diet alleviated these symptoms, they concluded that some unsaturated fatty acids were essential and must be supplied in the diet. Furthermore, the animal was apparently unable to synthesize these acids.

The work of Burr and Burr (5) contradicted the idea of Leathes and Wedell (9) that the animal body is capable of desaturating fatty acids. The oleic acid present in all animal fats would undoubtedly have been desaturated to linoleic acid and thus prevented the development of the deficiency symptoms had the animal possessed this power. However, Stetten and Schoenheimer (18) have shown by the use of deuterium that the animal can desaturate palmitic and stearic acids to palmitoleic and oleic acids, respectively. There has been no evidence to prove that the desaturation can progress further than oleic acid as no deuterium labeled linoleic acid was found. Thus, further evidence was presented which showed that the latter could not be synthesized in the animal body.

The relative potencies of the fatty acids in the cure of deficiency diseases have been studied by various workers. Hume et al. (7) found that linoleic acid was six times more

potent than linolenic acid in curing dermatitis and promoting growth. Burr (4) has stated generally that both are very effective curative agents. Quackenbush, Steenbock, Kummerow and Platz (15), on the other hand, found linolenic acid to be non-functional in reproduction.

Some of the B-complex vitamins have also been shown to be functional in fat metabolism (11). The effect of pyridoxine and pantothenic acid seems of special importance. Birch (1) concluded that pyridoxine was in some way connected with the unsaturated fatty acid factor of Burr and Burr (5) and therefore was important in fat metabolism. Furthermore, pyridoxine was discovered by Birch, Gyorgy and Harris (2) to be effective in protecting the rat from acrodynia. Schneider et al. (17) have suggested that pyridoxine and the essential fatty acids are interchangeable. However, Quackenbush et al. (15) have shown these conclusions to be invalid. Umbriet and Gunsalus (19) have suggested that pyridoxine is also concerned in amino acid metabolism.

Lippman (10) has recently shown that pantothenic acid can be added to the group of B-complex vitamins which function as coenzymes. For some time it has been realized that pantothenic acid protects the chick from a characteristic dermatitis in much the same manner as pyridoxine protects the rat. On the other hand, Quackenbush et al. (15) found that both pyridoxine and pantothenic acid were necessary for proper function of the unsaturated fatty acid factor. Their work indicated that the

two vitamins were functional, but they were unable to determine how these factors were involved in fatty acid metabolism.

Kummerow et al. (8) have found that the onset of rancidity was dependent on the presence of highly unsaturated fatty acids in the depot fats of turkeys and chickens held in cold storage for an extended period of time. Ethanolamine seems to be concerned in the overall metabolism; i.e., when ethanolamine was present the birds seemed to deposit less fat. The removal of linolenic acid seemed of particular value in the prevention of rancidity. Therefore, it would appear that ethanolamine, as well as the B-complex vitamins, may be concerned with fat metabolism.

Since some question exists regarding the biological effectiveness of linolenic acid, the present experiment was undertaken in an attempt to study the factors involved in the basic metabolism of the acid. Because rancidity of cold storage poultry is a great problem in the frozen food industry, a possible alteration of the deposited body fat is important. Linolenic acid metabolism is significant since it is conducive to rancidity development. This experiment was undertaken to clarify problems which are of interest to the frozen food industry and to nutritionists.

EXPERIMENTAL

Rats of the Sprague-Dawley strain, three weeks of age and weighing from 40 to 50 g, were used for the experiment. Each rat was weighed, transferred to an individual wire cage which was numbered, and given water and diet V ad libitum. The animals were kept in a constant temperature room at 28° C.

Diet V (Table 1) of Quackenbush, Platz and Steenbock (13), which is free of pyridoxine, pantothenic acid and fat, was prepared as follows: 200 mg of thiamin hydrochloride and 400 mg of riboflavin were dissolved in N/50 acetic acid, mixed with 18 pounds of casein, dried and then mixed with 78 pounds of cerelese and 4 pounds of Wesson salts in a standard feed mixer. The diet of the animals was supplemented once a week with one drop of fish liver oil containing 250 IU of vitamin A and 100 IU of vitamin D₃ per drop.

Animals kept on diet V developed severe dermal symptoms of fat deficiency or acrodynia in 7 to 8 weeks. The various stages of the dermal condition of each animal was recorded weekly by a modified method of Quackenbush et al. (13) as shown in Table 2. The dermal index was a numerical expression of the skin condition and was an indication of the condition of the eyes, ears, lips, fore paws, hind paws and tail. These parts of the body were the most acutely affected. Dermal index number increased with severity of the acrodynia.

The rats were divided into groups of three to six animals each and fed the supplements (Table 3) by calibrated medicine dropper. The ethyl linolenate was prepared from hexabromide according to the method of Rollet (16). The resulting product was distilled under high vacuum and kept under an atmosphere of oxygen at -13° C. Water soluble supplements were prepared by dissolving the synthetic vitamins in water. Ethanolamine, neutralized with concentrated hydrochloric acid and diluted with water, was used. After three weeks of supplementation, the animals were killed and extracted according to the method of Quackenbush and Steenbock (14). Iodine values of the resulting fatty acids were determined according to the method described by Yasuda (20). Composition of the mixed fatty acids was determined spectrophotometrically according to Brice et al. (3). All methods are outlined under experimental procedures.

Percentages of fatty acids as calculated from spectrophotometric analysis were converted to mg per 100 g. By considering the mean weight of fatty acid per 100 g, the resulting calculations reflected the difference more accurately than a comparison of percentage alone. The formula used for calculation of mg was as follows:

$$\frac{\% \text{ fatty acids}}{100 \text{ g}} = \frac{X}{\text{mean weight extracted fatty acid}}$$

$X = \text{gm fatty acids}$
 $X \times 1000 = \text{mg fatty acid}$

The amount of any one fatty acid synthesized or metabolized during the experimental period was determined by considering fat synthesis or metabolism of the control group. The amount of acid calculated for the control group was subtracted from all other groups. Results showing plus values indicated synthesis, minus values indicated metabolism.

EXPERIMENTAL PROCEDURES

Preparation of Supplements

Preparation of Linolenic Acid. Linolenic acid was prepared by heating 300 g of potassium hydroxide, 1200 ml of ethyl alcohol, and 100 ml of distilled water to nearly boiling on a steam bath. One thousand g of linseed oil was added to the hot solution and the entire mixture was refluxed for 30 minutes. The hydrolysate was cooled with tap water to about 40°C . and 1300 ml of cold distilled water was added. The solution was acidified with cold concentrated hydrochloric acid. The cold mixture was transferred to a large separatory funnel and shaken to decompose the soaps. After washing the fatty acids twice with water, one liter of ethyl ether was added and the fatty acids were washed again. When the water had been removed, the fatty acids were dried over sodium sulfate. One liter of ether was again added and the solution was allowed to stand overnight at -13°C . The sodium sulfate and saturated

acids were removed by filtering and another liter of ether was added to the filtrate which was ready for bromination.

Preparation of Crystalline Hexabromostearic Acid. The flask containing the fatty acids was clamped into an ice bath. A mechanical stirrer agitated the solution. Bromine was added slowly from a separatory funnel and the fatty acids were kept at low temperatures. About 580 g of bromine was required for complete saturation as determined by the persistence of the bromine color. The flask was allowed to cool overnight at -13° C. Crude crystalline hexabromostearic acid was collected on a Buchner funnel, washed with ether and transferred to a dry round bottom flask. Sufficient quantities of ether were added to insure complete solution and the mixture was refluxed on a steam bath. Twenty g of Norit was added, refluxing continued for a few minutes, then the hot solution was refiltered through a Buchner funnel. The filtrate was allowed to stand overnight at -13° C. and the product was filtered on a Buchner funnel and washed with ether. The white crystals were dried at room temperature.

Preparation of Ethyl Linolenate. Two hundred g of hexabromostearic acid and 200 g of zinc were mixed and put into a found bottom flask. Following the addition of 200 ml of ethyl alcohol and 2 ml of concentrated sulfuric acid, the solution was refluxed for two hours. The mixture was cooled under tap water and poured into a separatory funnel containing distilled water. After washing thoroughly, the mixture was allowed

to stand until the ester of linolenic acid separated from the water. The aqueous solution was removed and extracted twice with petroleum ether. The ester and the ether solutions were washed with sodium carbonate solution and water. The ether fraction was dried over sodium sulfate, filtered and freed from solvent under reduced pressure.

Saponification

The rats were killed with ether and allowed to digest on a steam bath with 100 cc of 30 percent potassium hydroxide per 100 gm of tissue. After six hours, one fifth volume of ethyl alcohol was added and the digestion continued for another two hours. The hydrolysate, freed from bones, was poured into Erlenmeyer flasks and refluxed for an additional two hours. The hydrolysate was then cooled and poured into large separatory funnels. The non-saponifiable material was removed by two extractions with one third volume of Skellysolve F and discarded. After removal of the non-saponifiable material, the solution was cooled under tap water and acidified with dilute 1:1 hydrochloric acid. The fatty acids were extracted three times with one third volumes of Skellysolve F. Crude fatty acids in the Skellysolve F layer were washed twice with distilled water to remove any excess hydrochloric acid and dried over anhydrous sodium sulfate. When the fatty acids had been partially freed from solvent under reduced pressure, the fatty

acids were poured into a weighed flask and the remaining solvent removed in a vacuum oven. Percentages of fatty acids were calculated when the tared flask and mixed fatty acids had been reweighed.

Iodine Value

Approximately 0.1 g of the mixed fatty acids was weighed into a glass stoppered iodine flask. Five ml of chloroform and exactly 15 ml of Wijs solution were added. The flasks were stoppered and placed in the dark. At the end of one hour, 10 ml of 15 percent potassium iodide solution was added and mixed thoroughly. The glass stopper and sides of the flask were rinsed with 10 ml of distilled water. The sample was titrated with N/10 sodium thiosulfate solution to a faint yellow. A few drops of starch solution were added and the mixture shaken well to free all the iodine. The solution was then titrated to a colorless end point. Two blank determinations were titrated with the unknowns.

Wijs Solution. Thirteen g of iodine was dissolved in a liter of glacial acetic acid. The solution was heated until all the iodine crystals were dissolved and then cooled. Chlorine gas was bubbled into the solution until the color was orange brown. The titration of the Wijs solution was double the titration of the original iodine solution when titrated with N/10 thiosulfate solution.

Potassium Iodide Solution. Fifteen g of potassium iodide was dissolved in 85 ml of distilled water.

N/10 sodium thiosulfate solution. Twenty four and eight tenths g of sodium thiosulfate were dissolved in one liter of distilled water and the exact normality determined by the following procedure: Exactly 10 ml of N/10 potassium dichromate was measured into an iodine flask and 5 ml of concentrated hydrochloric acid added. Ten ml of a 15 percent potassium iodide solution was added and the mixture was immediately titrated with thiosulfate solution to a greenish color. Starch solution was added and the titration continued slowly with occasional shaking until the solution turned bright, clear green in color.

The formulas used in calculating the iodine value were:

$$\text{Normality factor} = \frac{126.9 \times .1 \text{ (normality of dichromate)}}{\text{ml thiosulfate used}}$$

$$\text{Iodine value} = \frac{\text{normality factor} \times (\text{blank tit.} - \text{sample tit.})}{\text{sample weight}}$$

Spectrophotometric Analysis

Approximately 0.1 g of mixed fatty acids were pipetted into small weighing vessels. The vessels were placed in marked ignition tubes. Three ml of freshly prepared ethylene glycol containing 7.5 percent potassium hydroxide were added to each sample. Nitrogen was blown into each tube to replace air and the tubes were stoppered with glass tops and placed in a wire

basket. The basket was placed in preheated oil bath at 180° C. and the tubes held at this temperature for 30 minutes. Two blank determinations were run with each set of unknown samples.

After heating for 30 minutes, the basket was removed from the bath and the oil was wiped from the tubes with a piece of cloth. Contents of the tubes were transferred quantitatively to 100 ml volumetric flasks using 95 percent ethyl alcohol. The solution was diluted to 100 ml. Each sample was mixed. The volumetric flasks were allowed to stand overnight in the cold room so that the silica from the glass precipitated. The samples were removed from the cold room and allowed to stand until they reached room temperature. Each sample was filtered into an Erlenmeyer flask. A 10 ml aliquot was carefully transferred to a 250 ml volumetric flask, diluted to volume and mixed thoroughly. The samples were read on the Beckman spectrophotometer and readings were recorded at 2320, 2340, 2620, 2680, 2740, 3100, 3160 and 3220 \AA . If samples could not be read at the above wavelengths, suitable dilutions were made as previously described. Blanks were diluted by the same method as the unknowns.

Ethylene Glycol. Fifteen g of ground potassium hydroxide was dissolved in 180 cc of ethylene glycol.

The formulas used for calculating the fatty acid compositions were:

$$K_2 = \frac{K_{2320}}{\text{wt sample per liter}} - .04$$

$$K_3 = \frac{2.8}{\text{wt sample per liter}} (K_{2680} - K_{2620} - \frac{K_{2740}}{2})$$

$$K_4 = \frac{2.5}{\text{wt sample per liter}} (K_{3160} - K_{3100} - \frac{K_{3220}}{2})$$

The preceding formulas were those used for unisomerized samples which were used as correction factors.

$$K'_2 = \frac{K_{2320}}{\text{wt sample per liter}} + .07$$

$$K'_3 = \frac{4.1}{\text{wt sample per liter}} (K_{2680} - K_{2620} - \frac{K_{2740}}{2})$$

$$K'_4 = \frac{2.5}{\text{wt sample per liter}} (K_{3160} - K_{3100} - \frac{K_{3220}}{2})$$

where K_{2320} , K_{2620} etc. designated the spectrophotometric readings of the fat sample at that particular wave length.

$$K''_2 = K'_2 - K_2$$

$$K''_3 = K'_3 - K_3$$

$$K''_4 = K'_4 - K_4$$

$$\text{Percent linoleic acid} = 1.125 \times K''_2 - (1.27 \times K''_3) + .04 K''_4$$

$$\text{Percent linolenic acid} = 1.87 \times K''_3 - 4.43 \times K''_4$$

$$\text{Percent arachidonic acid} = 4.43 \times K''_4$$

$$\text{Percent oleic acid} = \text{Iodine number} \times 100 (181.5 \times \text{percent linoleic acid} - 273.5 \times \text{percent linolenic acid} - 333.5 \times \text{percent arachidonic acid}) / 90.$$

$$\text{Percent saturated acids} = 100 - \text{percent linoleic acid} - \text{percent linolenic acid} - \text{percent arachidonic acid} - \text{percent oleic acid}.$$

RESULTS

In all groups which did not receive pyridoxine and/or calcium pantothenate, a loss of body weight and aggravation of dermal condition was noted. When ethyl linolenate and ethanolamine were fed a loss of 13.5 gm mean body weight occurred. Ethanolamine alone caused a severe aggravation of the skin condition. The addition of pyridoxine and calcium pantothenate brought about large weight gains in all cases. Ethyl linolenate supplemented with the two vitamins produced a gain of 47 gm and caused improvement of the symptoms; ethyl linolenate alone caused a weight loss of 6.0 gm. Yet, when pyridoxine and calcium pantothenate were given without a source of essential fatty acids, no change in dermal index was noticed although the animals gained a considerable amount of weight.

Although the mean weight of mixed fatty acids extracted varied (Table 4), two trends were established. Without pyridoxine and calcium pantothenate, ethyl linolenate was a necessary factor for the deposition of fat. However, when choline or ethanolamine was given in combination with ethyl linolenate only 0.2 and 0.6 g fat respectively were extracted. Therefore, the possible role in metabolism of the two components of phospholipids might be suggested. The second trend established by the data showed that the amount of fat extracted increased greatly when pyridoxine and calcium pantothenate were

added. Ethyl linolenate with the two vitamins caused a deposition of 3.9 percent of fat.

Variation in the iodine value was great. Animals fed ethyl linolenate and tocopherol showed the largest and the group fed pyridoxine and calcium pantothenate with no fatty acid showed the smallest iodine value or 118.0 and 70.6, respectively.

Spectrophotometric analysis of the mixed fatty acids indicated large differences in composition of the deposited fat. Linolenic acid is of principal importance in this study. Table 5 indicates that groups receiving no supplement, ethanolamine, and tocopherol deposited no linolenic acid since there was no dietary source available. With linolenic acid, tocopherol showed the largest synthesis with 49.0 mg while linolenate alone showed 39.6 mg. Choline and ethanolamine seemed to bring about a reduction in the deposition of linolenic acid even though a dietary source was present.

When pyridoxine and calcium pantothenate were added to the diet the results were altered by the presence of the two vitamins. Except for one group no linolenic acid was found in spite of the dietary source. The animals receiving ethyl linolenate and only calcium pantothenate showed linolenic acid had been deposited in the tissue. However, when linolenate was fed with both vitamins all traces of this acid were removed.

As the linolenic acid fluctuated, arachidonic and lino-

leic acids showed changes. The greatest amount metabolized was indicated by the group which received linolenate and choline. In this group, however, the total percent of fat was low. The corresponding group with ethanolamine was also low, but a very slight synthesis was shown for both linoleic and arachidonic acids. Again, pyridoxine and calcium pantothenate seemed to be functional in the synthesis of these acids as shown by the group supplemented with the two vitamins and ethyl linolenate. This group synthesized 120.7 mg arachidonic acid and 64.3 mg linoleic acid over the control.

One of the largest variations was shown in a comparison of the amounts of oleic and saturated acids which were deposited. Previously mentioned trends were repeated. The combination of linolenate and choline effected a metabolism of 312 mg oleic acid, always common to the animal body, and 137 mg of saturated acid. Linolenate and ethanolamine, as has been shown with other acids, produced similar results but to a lesser degree. The addition of pyridoxine and calcium pantothenate caused large amounts of oleic and saturated acids to be synthesized. Animals receiving linolenate and the two vitamins produced 2188 mg oleic acid and 114 mg saturated acids. The group receiving only pyridoxine and calcium pantothenate also synthesized significant amounts of the two acids. The lower degree of unsaturation was in agreement with the lower iodine values of these groups. It is also interesting to note that the group receiving only linolenate and choline

had one of the highest iodine numbers and metabolized the largest amounts of oleic and saturated acids.

Table 1. Diet V.

Ingredients	:	Pounds/100# ration
Casein	:	18
Wesson salts	:	4
Cerelose	:	78
Thiamin hydrochloride	:	200 mg
Riboflavin	:	400 mg
Wesson salts		
Sodium chloride	:	105.00 gm/kg
Potassium chloride	:	120.00
Potassium hydrogen phosphate	:	310.00
Calcium diphosphate	:	149.00
Calcium carbonate	:	210.00
Magnesium sulfate	:	90.00
Ferric pyrophosphate	:	59.50
Manganese sulfate	:	.20
Potassium aluminum sulfate . $24\text{H}_2\text{O}$:	.09
Cupric sulfate . H_2O	:	.39
Potassium iodide	:	.05

Table 2. Stages of dermatitis.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Lips	Normal	Hyperemia, usually with small ulcers at corners	Slight swelling, redness along vertical groove	Medium swelling desquamation to nostrils	Severe swelling, raw sores around mouth and nostrils
Eyes	Very slight scaliness	Lids wet, slight crust at corners	Slight swelling of lids, crust in corners	Medium swelling, lids adhere with gummy exudate	Severe swelling, lids swollen shut, crusts on lids
Fore paws	Very slight scaliness	Slightly scaly redness, desquamation between digit	Slight swelling, digits desquamated and sore	Medium swelling, digits raw and sore, pus sores	Severe swelling, desquamated and sore, gangrenous
Hind paws	Same as fore paws	Same as fore paws	Same as fore paws, desquamation of large areas on paws	Same as fore paws, skin cracked and ulcerated	Severe swelling, large red areas on paws and inner thighs
Ears	Very slight swelling and slight scaliness	Hyperemia, slight swelling	Pinnæ swollen and thickened due to scale formation	Medium thickening at lips, large scales sloughing	Severe thickening, necrosis
Tail	Very slight scaliness	Scaly with small ringlets	Very scaly	Very scaly, necrosis near tip	Sections of tail may drop off

Table 3. The effect of supplements on dermal lesions and weight.

Group:	Supplements	Mean body weight change: in 3 weeks	Dermal index Initial: 3 weeks:	Description
I. Without pyridoxine and calcium pantothenate*				
1	None	- 7.0	5.0	7.5 Negative
2	18 mg ethanolemine	- 9.3	4.3	8.0 Negative
3	14 mg tocopherol**	- 8.3	4.3	8.0 Negative
4	87 mg et. linolenate	- 6.0	4.6	6.0 Negative
5	87 mg et. linolenate	-11.5	3.0	6.0 Negative
6	87 mg et. linolenate olemine	-13.5	4.0	5.5 Negative
7	87 mg et. linolenate erol**	- 6.0	3.6	5.0 Negative
II. With pyridoxine and calcium pantothenate*				
8	87 mg et. linolenate pyridoxine only	24.5	0.0	0.0 -
9	87 mg et. linolenate Ca pantothenate	12.0	5.3	5.5 Negative
10	None	41.0	4.3	4.3 No change
11	87 mg et. linolenate	47.0	4.0	2.6 Improvement
12	87 mg et. linolenate ethanolamine	32.6	6.4	2.2 Improvement

* 20 gamma pyridoxine and 50 gamma Ca pantothenate daily.

** Amount given weekly. Other supplements listed were given daily.

Table 4. The effect of supplements on total fat.

Group:	Supplements	Mixed fatty acids:		Iodine value
		Mean	Percent	
		Weight		
I. Without pyridoxine and calcium pantothenate				
1	None	.72	1.43	86.8
2	Ethanolamine	.69	1.29	94.5
3	Tocopherol	.68	1.13	85.2
4	Et. linolenate	1.32	1.95	97.3
5	Et. linolenate choline	.24	.44	115.4
6	Et. linolenate ethanolamine	.55	.88	105.9
7	Et. linolenate tocopherol	1.30	1.58	118.0
II. With pyridoxine and calcium pantothenate				
8	Et. linolenate pyridoxine only	3.71	6.31	88.2
9	Et. linolenate Ca pantothenate only	2.63	1.19	84.5
10	None	3.51	3.13	70.6
11	Et. linolenate	4.24	3.86	74.2
12	Et. linolenate ethanolamine	3.73	3.75	77.5

Table 5. Spectrophotometric studies of mixed fatty acids from carcasses of rats.

Group:	Supplements	:Arachidonic : Linolenic : Linoleic : Oleic : Saturated											
		: %	: Mg*	: %	: Mg*	: %	: Mg*	: %	: Mg*	: %	: Mg*	: %	: Mg*
I. Without pyridoxine and calcium pantothenate													
1	None	5.6	0	0	0	4.0	0	67.5	0	22.9	0		
2	Ethanolamine	7.0	12.3	0	0	5.5	9.1	67.9	-20	19.6	-27		
3	Tocopherol	5.2	-2.0	0	0	3.8	-3.0	67.6	-27	23.4	-2		
4	Et. linolenate	6.5	45.3	3.3	39.6	4.0	24.1	65.7	381	20.4	106		
5	Et. linolenate choline	7.8	-19.2	5.0	12.0	6.1	-14.4	71.8	-31.2	9.3	-137		
6	Et. linolenate ethanolamine	8.0	2.6	2.7	14.7	5.6	2.0	68.5	-108	15.2	-76		
7	Et. linolenate tocopherol	9.7	81.0	3.8	49.0	7.1	62.2	69.2	415	10.2	-28		
II. With pyridoxine and calcium pantothenate													
8	Et. linolenate B ₆ only	3.4	55.0	0	0	2.9	105.1	72.5	1201	21.1	399		
9	Et. linolenate Ca panto only	3.8	58.5	.89	23.1	3.2	54.4	71.2	1364	28.7	581		
10	None	1.0	-5.2	0	0	2.0	41.4	68.5	1860	27.8	824		
11	Et. linolenate	3.8	120.7	0	0	2.2	64.2	63.9	2188	30.0	1114		
12	Et. linolenate ethanolamine	3.4	86.5	0	0	1.8	38.3	69.5	2112	24.4	750		

* Milligrams of a fatty acid synthesized (plus values) or metabolized (minus values) as calculated from percent and mean weight of extracted fatty acids.

DISCUSSION

The biological effectiveness and metabolism of linolenic acid seem to be dependent on the presence of pyridoxine and pantothenic acid. The nutritive value of the various fatty acids has been discussed by Hume et al. (6). The relative potency of ethyl linolenate compared with other esters of fatty acids is not important here, but of significance is the improvement in dermal symptoms and weight. The general state and well being of the animals were markedly improved when linolenate, pyridoxine and calcium pantothenate were given together. These results are in general agreement with those found by Burr and Burr (5) who had added yeast, a rich source of both vitamins, to their basal diet.

Hume et al. (7) have concluded that linolenic acid is changed upon entering the body. Nunn and Smedley-MacLean (12) have further concluded that linolenic acid is not stored as such in the animal body unless large amounts are fed. These conclusions are again illustrated in the present experiment and here, as in the work of Nunn and Smedley-MacLean (12), pyridoxine and pantothenic acid were present. However, without the vitamins, linolenic acid evidently was not changed upon entering the body since the three double bond acid is found as such in the animal body fat.

Removal of linolenic acid seems not only of value in prolonging the onset of rancidity as suggested by Kummerow

et al. (8) but seems also essential for the life of the rat. When ethyl linolenate was given to the animals, two out of three died before the three weeks' experimental period had elapsed. Analysis of these animals showed that linolenic acid was present in extracted fat. Yet, when pyridoxine and calcium pantothenate were added with linolenate the deficiency disease was cured and the animals returned to normal. Analysis of the extracted fat showed no linolenic acid. Similar results were indicated when only linolenate and pyridoxine were fed, however, growth was slightly impaired without calcium pantothenate. Pyridoxine was evidently functional in removing the linolenic acid. Pyridoxine was also a stimulus to growth, although the weight gain was slightly sup-optimum.

As Burr and Burr (5) have suggested, a dietary source of unsaturated fatty acids must be provided. However, in the presence of pyridoxine and calcium pantothenate, the rat organism seems capable of converting the furnished fatty acids to other unsaturated acids such as linoleic and arachidonic acids. Nunn and Smedley-MacLean (12) have pointed out that unless linoleic or linolenic acids are present, the rat is not capable of synthesizing the more highly unsaturated and longer chain fatty acids which seem necessary for life. They further suggest that linolenic and/or linoleic acids function as building blocks for the higher acids. Furthermore, Nunn and Smedley-MacLean (12) suggested that linolenic acid is converted to a C_{22} acid with five double bonds. They believed that the

body accomplished the conversion not by desaturation but by building longer chains from linolenic acid, double bonds being formed in the process. The present data cannot confirm this suggestion since at present the spectrophotometric method of analysis does not include unsaturation higher than four double bonds. However, the idea seems possible in the presence of pyridoxine and pantothenic acid. This work does show that the vitamins and ethyl linolenate combine to synthesize considerable amounts of linoleic and arachidonic acids. The possibility that arachidonic acid is built as has been suggested for a C_{22} acid is feasible.

Quachenbush and Steenbock (14) were unable to explain lower iodine values obtained when rats were fed supplements of pyridoxine and calcium pantothenate. A partial hydrogenation catalyzed by the two vitamins appears to be one possible explanation. Yet, when the vitamins were given without a fatty acid, the iodine value was lowered. The possibility that pyridoxine and calcium pantothenate stimulated the production of fat from carbohydrate and/or protein also would seem logical. Determination of the mechanism by which the synthesis of the more saturated acids occurred was not possible here. However, after further investigation, the alteration of metabolism toward deposition of more saturated fatty acids might prove valuable as an approach to the delay of rancidity.

The function of choline and ethanolamine seemed to be

quite similar. In both cases, the supplements prevented the deposition of fat. When only ethanolamine was given the percentage of extracted fat was higher than when linolenate was supplied in the diet with ethanolamine. In some undetermined way the ethanolamine and choline evidently caused the fat to be metabolized. However, when the two were present, the smaller amount of fat which was deposited seemed to be of a more unsaturated nature. When ethanolamine, ethyl linolenate, pyridoxine and calcium pantothenate were given together a competition probably existed between the vitamins and the ethanolamine. The percentage of fat deposited was slightly lower, the iodine value slightly higher, and the fatty acid composition slightly more unsaturated than when ethanolamine was omitted from a similar group.

Hume et al. (7) have suggested that no significant results were obtained by including tocopherol in the diet. They state that skin lesions were healed and weight increases appeared to be promoted as well in the absence as in the presence of tocopherols. The present results show that vitamin E made no significant contribution to the state of the animal.

SUMMARY

Analysis of extracted carcass fat showed that linolenic acid was not deposited in the tissue when pyridoxine and calcium pantothenate were given with ethyl linolenate. However, without the two vitamins, linolenic acid was deposited and the animals died. On supplements of linolenate, pyridoxine and calcium pantothenate, the amounts of linoleic and arachidonic acids were higher than in groups not receiving the vitamins. A possible conversion of linolenic acid to linoleic and arachidonic acids was suggested,

Iodine values of groups receiving pyridoxine and calcium pantothenate were lower than those of groups not supplemented with the vitamins. Analysis of fatty acid composition showed more oleic and saturated acids had been deposited by rats receiving the two vitamins.

Chlorine and ethanolamine supplementation caused a lowering of the percentage of fat extracted. Although the amount of fat deposited was considerably less, a higher degree of unsaturation was noticed in agreement with higher iodine values. The results indicated a possible role of ethanolamine and choline in linolenic acid metabolism.

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